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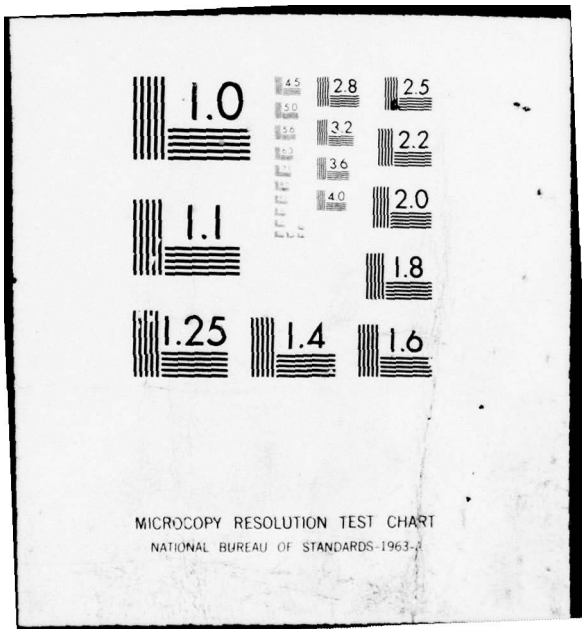
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER CI 79-55T	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* An Investigation to Determine the Practical Minimum Salt Requirements under Stress		5. TYPE OF REPORT & PERIOD COVERED 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Bruce Bland Banias		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS AFIT Studnet at the University of Southern California		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS AFIT/CI WPAFB OH 45433		12. REPORT DATE August 1978 13. NUMBER OF PAGES 59
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for Public Release, Distribution Unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

APPROVED FOR PUBLIC RELEASE AFR 190-17.

JOSEPH P. HIPPS, Major, USAF
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18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

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SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

79-55T

(6) AN INVESTIGATION TO DETERMINE THE PRACTICAL MINIMUM SALT REQUIREMENTS UNDER STRESS.

by

(10) Bruce Bland Banias

(9) Master's thesis,

(14) AFIT-CI-79-55T (12) 67 p.

A Thesis Presented to the

FACULTY OF THE GRADUATE SCHOOL

UNIVERSITY OF SOUTHERN CALIFORNIA

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

(Physiology)

(11) August 1978

UNIVERSITY OF SOUTHERN CALIFORNIA
THE GRADUATE SCHOOL
UNIVERSITY PARK
LOS ANGELES, CALIFORNIA 90007

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MASTER OF SCIENCE

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ACKNOWLEDGMENTS

I would like to take this opportunity to thank those who made this project possible. Thanks to Dr. James P. Henry for his patience, his articulate sense of humor, and his understanding towards me as a person. I would like to express my deepest gratitude to Patricia Stephens for her magic feet, her ability to stabilize a situation in all forms of anxiety, and the fact that through it all, she never lost sight of the silver lining. To Paul Meehan, I would like to express my unending friendship in appreciation for his support, his help, and for just being there when I needed understanding. To Jo Kolsum, I would like to say thank you for showing me what unending patience is. Most of all, I would like to thank my wife and family. Without their help and support I would never have been able to be where I am and be doing what I am doing today. I also extend praise to my Lord Jesus Christ for giving me the strength to be here and for watching over me and my family in this time of pressure as well as pleasure.

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INTRODUCTION

It has been established that mice placed in a psychosocially stressful situation will develop hypertension in the same way it develops in man (29). In recent years, the control of hypertension has been the subject of many an investigation.

Today, a widely used approach in controlling high blood pressure is the restriction of salt intake. The Kempner diet, for example, is sufficiently low in sodium (150 mg/day) to be effective, but it is unpalatable. It not only has a flat taste, but it also has accompanying anorexia, weakness, and impairment of memory during the first few weeks on the diet, resulting in sufficient malaise to make patient acceptance poor.

The use of thiazide diuretics is an alternative method of controlling body sodium. It is so widespread that some physicians are not too concerned about their patients' intake of sodium if they are on diuretics.

Since a suitable diet for mice had to be established before salt intake could be evaluated as a potential influence in psychosocially induced hypertension, the goal

of this pilot study was to determine the necessary ingredients for a nutritionally balanced diet in which the level of sodium could be easily controlled.

LITERATURE REVIEW

Early Work

As early as 1922 the reduction of sodium in a hypertensive's diet was shown to cause a reduction in blood pressure (2,3). This evidence led many investigators to ask what was the appropriate daily salt intake requirement of a normal man as well as what were the effects of modifying this intake on blood pressure.

In the early 1940's, Kempner developed a rice diet which satisfied the caloric and nutritional requirements of a man at rest; yet, it contained only 150 milligrams of sodium. This was less than one-half gram of salt a day in total intake. Kempner observed that patients on this diet had a remarkable reduction of blood pressure; however, unless remaining quietly in bed, they complained of mild dizziness upon standing, weakness, and a slight impairment of memory. These symptoms are in keeping with the picture of sodium depletion (33).

An epidemiologic approach to salt and blood pressure looks at populations that have thrived on low salt diets and have remained normotensive throughout their lives. In

a current review, Fries states that epidemiological studies in unacculturated people show that the prevalence of hypertension is inversely correlated with the degree of their salt intake (24). He argues that this is no coincidence.

In the investigation of this hypothesis, the question of how much sodium chloride a man needs to maintain health, not while in bed, but under customary social interactions, must be answered. Dahl observed that among unacculturated people with low blood pressure, it is rare to find a group with a sodium chloride intake exceeding 4 to 5 grams a day (14). He cited Kempner's contention that a person could subsist on a salt intake of less than one gram a day; however, in practice, this is feasible only if sweating is avoided. Even Dahl suggested that the daily intake be kept between 1 and 2 grams (15).

To achieve the minimum salt intake (1 to 2 grams/day), unacculturated peoples have made certain adjustments in their life style and in their food source. In their work with the Yanomamo Indians, Oliver et al. (48) reported a urinary sodium output of just 1 to 3 milliequivalents/liter/day. At face value, this intake was less than one-fifth gram of salt a day. However, the work of Chagnon (unpublished data) indicated that in the groups he studied, each man, woman, and child had a fish plus meat intake equivalent to 400 grams a day. Thus, their sodium

consumption equivalent was 1 1/2 grams of salt a day in a 70 kilogram man. If Chagnon was correct, we can only assume that the Yanomamo group Oliver observed suspended hunting and fishing during this study. As was expected of such relatively heavy meat eaters, they were not driven to get additional salt by trading; however, they practiced an endocannibalistic ritual of eating the powdered bones of their dead. This added significantly to their daily sodium intake because bone contains 40% of the body sodium.

In contrast to the Yanomamo, some people who were not heavy meat eaters developed an extensive salt trade. An example given by Meggitt was the diet of the natives of the western highlands of New Guinea (40) which consisted mostly of the roots of tuberous plants and some pork. Possibly these people developed this salt trade to compensate for a dietary deficiency of salt. Interestingly, their salt intake greatly increased during rituals associated with violent exercise and warfare.

In contrast to the foregoing studies, there seemed to be no deleterious effect from the moderate consumption of salt. Indeed, many persons throughout the world remained normotensive despite a high intake. The suspicion that only consumption and individual differences were responsible for essential hypertension in urbanized cultures overlooked the possible role of stress.

Certainly Selye (57) long ago provided a convincing demonstration that high levels of adrenal-cortical activity (associated with high levels of perceived stress) and salt intake levels influence the onset of hypertension. Through the administration of desoxycorticosteroid acetate (DCA) he mimicked high adrenal-cortical activity. When the administration of DCA was combined with high levels of salt intake, malignant hypertension developed; this, answered the question that salt intake and stress levels can be related.

Human studies showed that patients must use less than 2 grams of salt a day to get the benefit of reduced blood pressure while taking DCA. While not experiencing the malaise of Kempner's patients, they experienced salt hunger during stress induced adrenal-cortical activation.

Along these lines, Aumann and Emlin's work with Microtine rodents (voles) conclusively demonstrated that as stress loads rose, so did salt hunger (5). Drawing on Christian's research showing as population density of a territorial animal colony increased likewise, the adrenal-cortical activity increased. They offered voles plain water or water with sodium chloride in an experimental population cage. Non-stressed voles chose plain water, but as the stress level increased by the addition of animals to the cages, they resorted to drinking more water with sodium

chloride until it was 90% of their fluid intake.

The data suggested that a high salt diet greatly increased the risk of hypertension developing in persons with high adrenal-cortical activity. On the other hand, a diet sufficiently low in salt had a protective effect.

Salt Intake in Our Society

Food companies, through the addition of salt to all but a few processed foods, made the selection of a diet which contained 2 to 3 grams of salt a day impossible. The manufacturers even added extra salt to baby food so it tasted good to the mother. Consequently, we easily consumed more than 15 grams a day when using these foods instead of fresh products (26). Such eating habits increased a normotensive's blood pressure 10 mmHg. But, patients on a thiazide diuretic continued on a regular diet while maintaining the body sodium level of only 2 grams of salt a day (22). When salt intake was kept to less than 10 grams a day, thiazide diuretics regularly reduced systolic blood pressure by 20 mmHg.

High Blood Pressure Mechanisms and Salt

It was suggested that blood pressure rises in order to excrete sodium when the capacities of the other control systems are exceeded (41). Also, there was evidence that a low level of salt in the body caused norepinephrine changes in the peripheral nervous system. Body sodium and water

appeared to be important in the determination of storage and turnover of norepinephrine (38). Perhaps, then, the effectiveness of diuretic drugs depended upon having a moderate daily intake of sodium chloride. Kirkendall et al. showed that for maximum reduction in blood pressure with a diuretic, it was necessary to have a daily salt intake of 4 to 6 grams (36). A direct relationship was demonstrated between the sodium intake and the sensitivity of the sympathetic system (12,27). Indeed, the data, in general, showed that with an increased level of exchangeable sodium above the body's physiological need, there was a stronger vasoconstriction of blood vessels in the vascular bed. The opposite was true with a low level of exchangeable body sodium.

The renin-angiotensin system was implicated in sodium homeostasis and thereby became an important part of the blood pressure system. Bruner demonstrated that when sodium levels are depleted, the pressor response to angiotensin II was not as high as it was in a sodium-loaded animal (10). Thus, although sodium depletion increased the amount of angiotensin II in circulation by increased production of renin, the expected pressor response to this increase was not observed. This allowed the renin-angiotensin system to respond to low sodium levels without a concomitant rise in peripheral resistance, thus eliminating the expected rise in blood pressure.

It was pertinent to look at the influence of sodium intake on possible changes in vascular activity. Salt loading and salt deprivation was shown to alter the sensitivity of the vascular bed to norepinephrine (1). These two altered hormonal responses accounted for the decreased responsiveness of the vascular bed and thus explained the role of sodium in the maintenance of vasomotor control.

Blood pressure was influenced by differences in renal blood flow. Hollenberg compared renal blood flow in patients on a low salt diet with those on a normal or high salt diet and found that the different dietary regimes affected blood flow characteristics of the kidneys (30). He suggested that the long nephrons associated with the innercortical glomeruli were especially efficient in removing filtered sodium. These nephrons received more blood in a sodium depleted patient and supported the belief that maximum sodium retention was possible without a change in glomerular filtration rate.

A further feedback loop involved the macula densa, which was influenced by the amount of sodium in the plasma as the filtrate flowed down the tubules. The macula densa, in turn, controlled renin secretion, which regulated the production of aldosterone. It was observed that the sodium depleted patient not only had a rise in angiotensin II, but also in aldosterone (47). Thus, aldosterone secretion by

the adrenal cortex related to body sodium via the latter's relation to the macula densa.

Conclusions

A further study consolidating the concepts presented thus far showed that sodium intake influences both vascular and adrenal responses to angiotensin II (31). When sodium was restricted, the vascular response to angiotensin II was less than normal, while the adrenal response to angiotensin II was increased greatly. An increased level of aldosterone influenced the kidneys, which helped to retain the already low levels of sodium. The blunted response of the vascular bed to increased levels of angiotensin II prevented an increase in peripheral resistance and, therefore, an increased blood pressure.

Under opposite conditions of excessive sodium, Hollenberg found opposite results (31). The sodium loaded individual had a decreased level of renin-angiotensin II and a high level of body sodium. This allowed for an increased sensitivity to sympathetic release at the vascular bed, a venous capacitance reduction, and a greater arterial constriction. This increased peripheral resistance allowed for an increased total blood pressure. The low levels of angiotensin II diminished the adrenal production of aldosterone, and these reduced levels in the kidney diverted the blood flow from the long nephrons of

sodium retention to the short nephrons which allowed the excess sodium to be excreted.

As increased levels of sodium were associated with increased vascular vasoconstriction, thus higher blood pressure, it followed that overloading the body with sodium was undesirable. The ideal was to use 2 to 4 grams a day to get the optimum hypotensive benefits from low salts. This intake was no lower than that of a predominately flesh eating people, such as the Eskimos. In such peoples, salt hunger was avoided and the extra salt demanded by sweating during exertion was just met. This intake avoided the problems that accompany a truly low sodium diet, such as the Kempner diet, and yet was low enough to derive at least some of the benefits of complex changes affecting vascular reactivity due to reduced sodium.

It was possible that psychosocial stress increased this requirement to perhaps as much as 4 to 6 grams a day. Indirect evidence pointing to this hypothesis was the rise in voluntary salt consumption among hypertensives (49). The heavy salt consumption of New Guinea warriors during rituals and warfare and the preference of voles for salty water instead of plain water during competitive interaction supported this theory. Perhaps during urbanization, adrenal cortical activity as a whole rose, and for this reason, the appetite for salt increased. A method of further study was

to contrast the survival rate of stressed and nonstressed animals on diets of varying salt content. This was the focus of the present study in various states of socially stressful situations.

METHODS AND MATERIALS

Experimental Animals

CBA agouti mice, a strain similar to the common house mouse, Mus musculus, were studied. Originally obtained from the Jackson Laboratory, Bar Harbor, Maine, these mice were raised at this laboratory for the past 23 years. Intercommunicating box systems or population cages were stocked with mice of the same age and approximate weight from several cages of socialized siblings. The term, socialized, referred to mice kept in cages with siblings of the same sex, i.e., not isolated at weaning. Thirty-two mice (16 males and 16 females) formed the nucleus of the colony. Since the success of the study depended on the length of time the colony survived, it was terminated when half of the males had died or when fewer males but several of the females had died. See Table 2 for termination data. Mice were identified by a code of ear punches.

Population Cages

The circular intercommunicating box system was designed for developing hypertension in CBA mice over an established period of time at the Experimental Physiology

Laboratories, University of Southern California (29). As shown in Figure 1, the system consisted of six plexiglass boxes (or cages). Each had three entrances or exits, two leading to other boxes in the system and the third to a central box containing food and water. Food was placed in a large quarter-inch mesh holder and the water in bottles. This system fostered psychosocial stress because mice are unable to establish and to defend territory normally; they fought constantly in a vain attempt to establish boundaries which were constantly trespassed by other mice.

Feeding Devices

A standard wire mesh basket was used for most of the colonies. The 3-inch diameter cylinder of quarter-inch hardware cloth was suspended 2 inches above the floor in the central food box. The size of the mesh allowed the mice to easily eat the standard laboratory mouse food (pellets). It was theorized, however, that mice on Purina test diets No. 5881 and No. 5775 were not getting enough to eat because of a change in the consistency and shape of the pellets. Therefore, another feeder was devised. Inside the wire mesh in the central box, a cylindrical plexiglass hopper with four 1-inch square holes at the bottom was attached to a plexiglass plate, and the unit was placed on the floor of the box. The food was broken up into small chunks for easy removal. Its weight was

sufficient to ensure a constant flow into the dish below. The wire mesh was not removed because it provided the mice a means of access to the spouts of the drinking water bottle. When the diet was changed to flat rolled grain, the original wire mesh basket was used again, allowing the suspended food to be removed easily by the mice.

Diets

Various types of diets were used in this pilot study. The first was the Hartroft diet, an artificial non-grain based mixture without salt, made by U.S. Biochemical Corporation, Cleveland, Ohio. Since this classic no salt diet was widely used for many years, its choice was logical for beginning the investigation into reduced salt intake and survival rates on mice during psychosocial stress.

The Ralston Purina Company of St. Louis, Missouri provided two artificial non-grain test diets with sodium added: No. 5775 with 0.29% sodium and No. 5881 with 0.05% sodium. These diets were white, cylindrical, and because of their hard consistency tended to break into sharp fragments when chewed. The standard Purina Lab Chow in contrast was brown, oval pellets that tended to crumble into small chunks when chewed.

After the tests were run on the artificial diets, it was felt that a natural grain based diet should be used. Grains then were selected for their natural lack of sodium

(see Table 3) and the final mixture of grains provided what was thought to be a balanced diet with minimum sodium content. As the grains in their natural state were not compatible with the standard wire mesh feeder, rolled grain products were used. The rolled grain products did not alter the sodium content or the grain mixture.

Finally, in an attempt to achieve a mixture as close to the standard Purina Lab Chow as possible, a new diet was ordered. This new diet from Purina was the standard Lab Chow with the maximum amount of sodium removed. This was accomplished by the addition of low-salt corn and soybean meal to the mix. The sodium content was reported by Purina to be 0.122%. Upon receipt of the new diet, acceptability testing was begun.

Social Stress Procedures

It was important in a study of this nature to be able to judge the amount of social stress placed upon the animals by the different housing environments. It was possible to estimate this stress by taking advantage of previous work using the adrenal medullary enzyme, tyrosine hydroxylase (TyOH) as a marker (29). (TyOH is the catecholamine synthetic enzyme responsible for norepinephrine.)

Isolated animals had no social contact with other mice and, therefore, very low levels of renin and TyOH.

They served as the base line for social stress, i.e., rating 0 (see Table 1). The minimal social stress load of young boxed male siblings was established as a slightly higher level of ±. The ± rating was also given to the boxed siblings used as growth study animals. The level of renin in these animals was higher than in the isolates (60) and dominance was often detected. When animals from different litters were used, there was fighting, and the renin, TyOH, and blood pressure rose (29,60). A score of + was given such non-sibling or mixed male animals.

When a colony living in a complex population cage was made up of socialized or boxed siblings, the ++ rating was used because in this situation these animals fought vigorously. A chronic sustained elevation of blood pressure and subsequent hormonal changes resulted. The highest rating of +++ was given when a colony was composed of animals that had been isolated for a full four months. Such colonies were not used in this study, but much work had been done with them (60).

This rating system permitted an approximate quantification of the social stress placed upon the animals and the results demonstrated that the effects of low sodium diets depended on the stress load of the animals.

Determination of Growth Curves

A growth study using isolate animals was begun by

first choosing ten mice at weaning age (3 to 4 weeks). They were placed in ten separate one quart mason jars and raised with an independent water supply and food ad lib until adulthood. The average weekly weight gain was plotted against time and a growth curve was established.

Boxed sibling animals were studied in a similar way. A large litter that contained at least eight males was used. These mice were raised communally in a standard polycarbonate box by two or three mothers. At weaning age (3 to 4 weeks) they were placed in another box and given food and water ad lib. Weekly weights were taken and the average weight gain versus time was plotted. The mixed males were handled similarly, but the selection differed. Here, ten animals were chosen from ten different sets of parents. At weaning they were placed together in a standard box. The weights were taken and a growth curve was established.

Each colony had an accompanying set of control mice of equal age and weight. The control mice were fed the same sodium content and composition food as their respective colonies. Weighings every two weeks ensured that they were maintaining health.

The animals used in the colony experiments were chosen from boxed siblings of approximately the same weight and age. This ensured that they did not have any significant

size advantage over one another when the colony was begun. Growth curve studies were not made in this high social stress situation, because the mice were adults when placed in the colony.

RESULTS

Blood Pressure Data

Since one of the primary tasks of this study was to determine whether sodium had an effect on blood pressure, blood pressure was measured every two weeks according to an established laboratory procedure (29). However, a measurement problem soon developed. The tail pulse of the mice that were on the low sodium diet declined to the point that the blood pressure could not be accurately measured. Therefore, this measurement was discontinued, despite its potential value.

Diets Used

This investigation used the following diets: 1) the classic Hartroft diet (no salt), 2) Purina test diet No. 5881 (0.05% sodium), 3) Purina test diet No. 5775 (0.29% sodium), 4) low salt grain mixture plus 0.3% sodium in the drinking water, 5) low salt grain mixture plus 0.08% sodium in the drinking water.

The results described were summarized in Table 2. They showed that although the Purina test diets with the additional sodium increased the life expectancy under high

stress load (stress ++), the diet was in some way inadequate because the colony animals did not live as long as those on normal Purina Lab Chow. Likewise, the grain diet was not acceptable. Here, although the diet was adequate for stress loads below +, the addition of extra sodium did not improve the life span of the animals.

Hartroft Diet

Isolates (stress 0) and boxed siblings (stress ±) were put on the classic Hartroft diet and their daily weight recorded. This diet consisted of 2% cellufil, 7% corn oil, 4% sodium-free salt mix, 66.1% sucrose, and 20% vitamin-free casein. The mice lost weight from the first day on this regimen. Although they averaged 35 grams at the outset, by the eighth day of the experiment they weighed about 20 grams and were either dead or dying. It should be remembered that this diet contained no sodium chloride.

Purina Test Diet No. 5881

Purina test diet No. 5881 was specially formulated to make the control of sodium intake easy. Ralston Purina Company asserted that only 0.05% of the diet was sodium. This is the equivalent in man to slightly more than one gram of salt a day.

A growth curve on this diet (Figure 2A) compared favorable with a growth study on the standard Purina Lab

Chow (Figure 2B). The lack of a significant difference between the curves confirmed Purina's assertion that the diet was adequate for nonstressed mice.

Socialized mice in boxes (stress ±) were also tested. The growth curves were not significantly different from those already established for isolated mice (Figure 3A).

Three boxes of fully adult control mice (stress ±) also on this diet, did not lose weight; but rather showed a slow, steady weight gain. These results further supported Ralston Purina's assertion that the diet was adequate for maintaining animals.

Additional stress was introduced by placing nonsibling males (stress +) together in a box and measuring their growth. As shown in Figure 3B, there was no significant difference in growth rate between the nonsibling males and isolates. This indicated that minorstress did not affect the adequacy of the diet.

Since increased stress was required to develop hypertension, the mice were placed in an intercommunicating box system and started on Purina test diet No. 5881 (stress ++). They were weighed and their blood pressures were taken at regular intervals during the life of the colony. The life expectancy of this colony proved to be low (Figure 4A) and was only prolonged by the addition of sodium to the water. The asterisks on the figure (Figure

4A) indicated the changed sodium strength.

This was the first high stress (stress ++) colony to be run on low salt. It was originally set at the equivalent in man of one gram a day. The many losses by cannibalization led to the addition of some sodium in an attempt to save the colony. It was thought that perhaps the combined effect of a sudden lack of sodium and intense social interaction might have led to the breakdown. When a 1.5 gram equivalent of sodium was added, making the total a 2.5 gram equivalent, there was a period of nearly two weeks without deaths. The additional sodium was then taken away. The high death rate resumed until the sodium was once more restored to the 2 1/2 gram level. However, after another ten days of what would have been adequate sodium levels for boxed siblings (stress ±), the mortality resumed. Thus, it was then realized that although there was sufficient sodium for boxed stress level + animals, either the diet or the sodium level or both were inadequate for animals in a stress ++ colony situation.

Prior to the termination of this first colony, two new colonies were started at the same sodium levels as the first and this level was not varied. More than half of the males were cannibalized in these new colonies (stress ++) in the first 20 days (Figure 4B). In an attempt to establish if the mice were starving to death, a new feeder

was put into the food box. The food was broken up into smaller more manageable chunks, but still the cannibalization occurred.

At autopsy, the adrenals, heart, and kidneys were weighed. No significant differences were found between these weights and the weights for a colony fed on standard Purina Lab Chow.

Purina Test Diet No. 5775

Purina test diet No. 5775 was specially formulated the same as diet No. 5881 discussed above, except that it had a sodium content of 0.29%. This was approximately equal to that of standard Purina Lab Chow, i.e., to 10 grams of salt a day in a 70-kilogram man.

A growth curve using isolated animals (stress 0) was established (Figure 2C). It was not significantly different from those for animals on the low sodium Purina test diet No. 5881 or the standard Purina Lab Chow.

The controls (stress ±) on this diet grew at a normal rate. During the 12 weeks they were observed, the controls grew much heavier than the controls on the Purina test diet No. 5881. At autopsy it was found that much of the weight gain was due to the accumulation of fat. The data confirmed Ralston Purina's assertion that test diet No. 5775 was adequate for maintaining mice.

A socialized colony was put on this diet (stress ++)

and the body weights and blood pressure were measured every two weeks. In contrast to the colony on a low sodium diet, these mice did quite well for 90 days; however, at this point, the first cannibalized animal was found. During the next 14 days, more than half of the males were cannibalized and the colony was then terminated (Figure 4C). As was the case with the mice on the low sodium Purina test diet No. 5881, these animals did not look or behave like normal, healthy CBA mice. They did not have the characteristic firm skin, glossy coat, and clear eyes. They were more irritable and developed tremors. These mice were also more sensitive to stimulation than the mice on the standard Purina Lab Chow. It should be mentioned that cannibalization does not occur with any frequency even in stress +++ colonies fed standard Purina Lab Chow.

Selection of the Grain Mixture

Since the low sodium Purina diet clearly did not work out in a high stress situation (stress ++), it was felt that a dietary change should be made. According to theory, a basic grain diet would be closer to the normal diet of a wild mouse; therefore, commercially available grains that were most readily eaten by mice were selected and tested. Groups of boxed mice were placed on different single grain diets to determine urinary sodium output (Table 3). In each case, urinary sodium output was less than 5 milli-equivalents per liter, and when the grains were combined,

the urinary sodium output established itself at 4 milliequivalents per liter. Rolled oats, rolled rye, and soybean chips were retained for the study. This combination of grains gave such a low urinary sodium output that the daily sodium intake was readily controlled by the addition of sodium to the drinking water.

Mixed Grain Diet and Tap Water

Both socialized and isolated mice were put on the mixed grain diet and tap water. Although they appeared to be unhealthy, no growth defects or gross pathology was observed. They had dull coats, poor skin texture, and were extremely irritable.

The controls (stress ±) on this diet did not lose weight and the isolated mice (stress 0) showed a slow, steady weight gain beyond 35 grams (Figure 2D). This was in contrast to socialized siblings (stress ±) in boxes that just maintained their weight. This data indicated that the mixed grain diet was adequate for the growth and maintenance of mice. But, a significantly high number of females in the breeder boxes died after giving birth to pups. These pups were smaller and required a longer time for successful weaning. This breeding problem contrasted unfavorably with that of mice on the standard Purina Lab Chow.

Two population cage colonies (stress ++) were put on

the mixed grain diet and no additional sodium was added to their drinking water. In both, more than half of the males were cannibalized by the twentieth day of the experiment (Figure 5A). This indicated that the mixed grain diet without additional sodium was not sufficient for survival under stress.

Mixed Grain Diet and Water with 0.3% Sodium

Control mice living in boxes (stress ±) did well on the regimen of mixed grain and 0.3% sodium in the drinking water. They demonstrated a constant weight gain and did not reflect the obvious health deficiencies that were seen in the mouse colonies.

In a stress study, a colony (stress ++) was put on the mixed grain diet and water with 0.3% sodium content. Although these mice appeared healthier than those on the Purina test diets, they were not as fit as those on the standard Purina Lab Chow. The colony's death rate was higher than that of a colony on the same mixed grain diet and 0.08% sodium or a colony on Purina test diet No. 5775 (compare Figure 5C to Figure 4C).

This colony was terminated when more than half of the males and two of the females were cannibalized. The heart, adrenals, and kidneys were weighed at autopsy and no significant deviation from the normal values of mice on the standard Purina Lab Chow were observed.

Mixed Grain Diet and Water with 0.08% Sodium

A 0.08% content of sodium represented the addition of 30 milliequivalents of sodium to the water. As already confirmed in the literature, 15 milliequivalents of sodium a day equaled a daily intake of 1 gram of salt by a 70 kilogram man; therefore, the addition of 0.08% sodium to the water was the same as an intake of 2 grams of salt a day.

The controls in boxes (stress ±) had no problems on this diet. They gained weight and appeared to be almost as healthy as others on the standard Purina Lab Chow.

The mixed grain diet with 0.08% sodium added to the drinking water was given to a stress colony (stress ++) to determine whether the increased sodium would produce different results from those obtained on the low sodium Purina test diet. This colony did indeed have a lower death rate than the stress colony on the mixed grain diet with 0.3% sodium and the colony on the low sodium Purina test diet (Figure 5C). The major difference, however, was that the females had an extremely high death rate and more than half were cannibalized or died during the period of observation.

Mixed Grain Diet and Water with 0.08% Sodium/ Peripheral

A colony was put on the grain mixture with 0.08%

sodium added to the drinking water, but the stress of competition for the centrally located food and water was diminished by placing food and water in each of the six peripheral boxes or cages comprising the circular intercommunication box system (Figure 1). The results of this investigation were more rewarding than the others previously mentioned.

The losses in this colony were by slow attrition rather than by massive cannibalism. Judging from the results of previous blood pressure studies and the logistics of the design, the level of stress was intermediate between that attained in boxed mixed males (stress +) and that in a colony (stress ++) with centrally located food and water.

TABLE 1

EXPERIMENTAL PROCEDURES FOR INDUCING SOCIAL STRESS RATINGS

Housing Environment	Social Stress Rating
Isolate	0
Boxed Siblings	±
Control Boxed Siblings	±
Boxed Mixed Males	+
Colony of Boxed Siblings	++
Colony of Isolates	+++

TABLE 2

SUMMARY OF RESULTS

Diets	Survivors* Males	Females	% of Normal (180 days)
Purina			
No. 5881 + water	6	16	11%
No. 5881 + 0.1% Na in water	2	14	44%
No. 5775 + water	7	14	58%
Mixed Grain			
Tap water	7	15	11%
Water + 0.08% sodium	12	6	33%
Water + 0.3% sodium	6	14	33%

All diets provided adequate growth and maintenance of unstressed animals.

*16 males and 16 females in each experiment.

TABLE 3

SODIUM LEVELS IN VARIOUS GRAINS

Measured by Urinary sodium output	
1. Purina No. 5881	17 mEq/Liter
2. Purina No. 5775	251+ mEq/Liter
3. Oats	1.0 mEq/Liter
4. Hard red wheat	1.2 mEq/Liter
5. Rye	3.0 mEq/Liter
6. Long grain rice	1.8 mEq/Liter
7. Soybean chips	2.0 mEq/Liter
8. Mixture of 3, 5, and 7	4.0 mEq/Liter

Figure 1.--This intercommunicating cage system is used to induce social interaction in mice and is usually stocked with 16 males and 16 females. The lucite cages, a standard vivarium (shoebox) size of 29 x 18 x 13 centimeters, are connected into a circle by flexible plastic tubes of 3.8 centimeter I.D. The central hexagon holds food and water and is connected to each cage by short tubes of 3.2 centimeter I.D.

Figure 2.--Growth Curves with Test Diets.

Figure A. Purina 5881 (low sodium) .05% Na (Isolates)

Figure B. Purina Standard Lab Chow 0.3% Na (Isolates)

Figure C. Purina 5775 (normal sodium) .29% Na (Isolates)

Figure D. Grain Mixture 0.01% Na (Isolates)

GROWTH CURVES WITH TEST DIETS

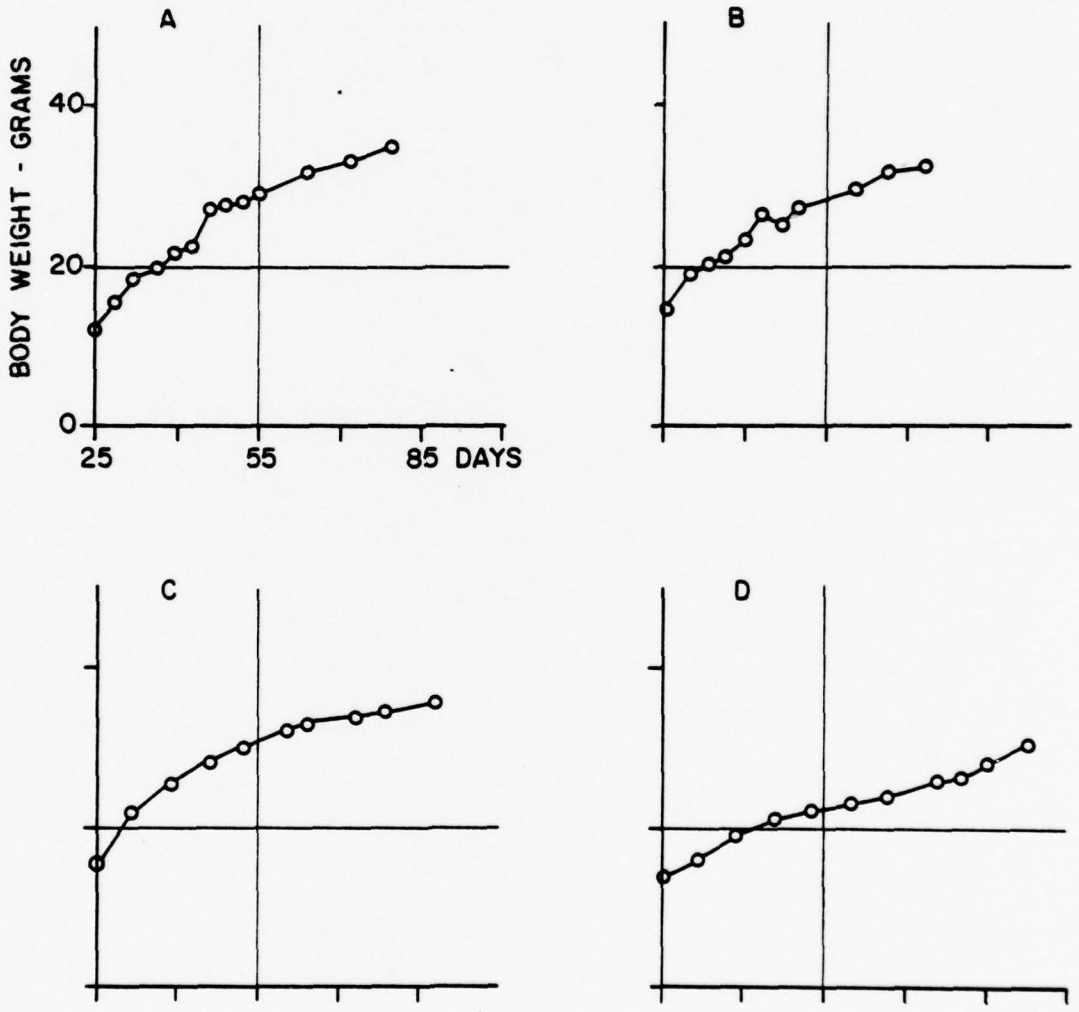


Figure 3.--Growth Curves Boxed Animals

Figure A. Boxed Siblings on Purina 5881 (low sodium)

Figure B. Boxed Mixed Males on Purina 5881 (low sodium)

BOXED ANIMAL GROWTH CURVES

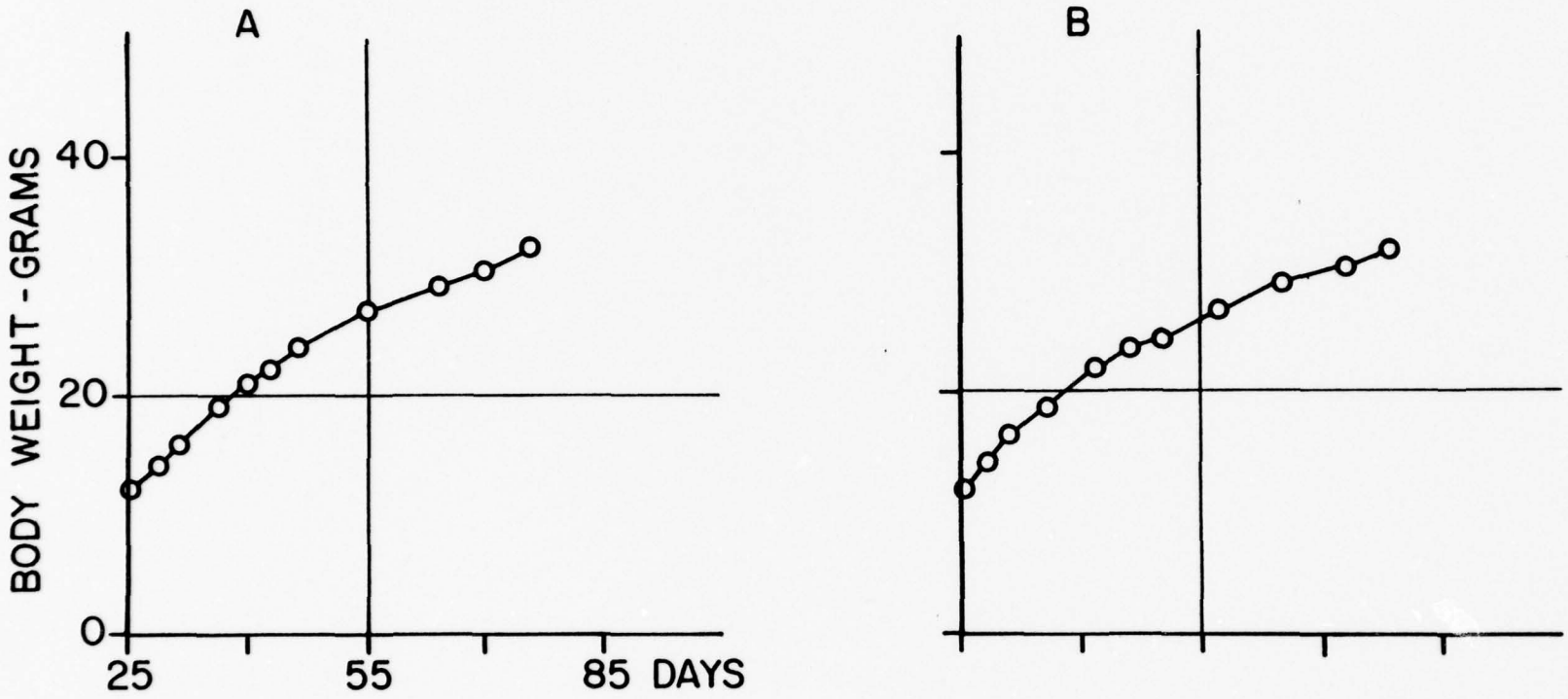


Figure 4.--Death Rates on Purina Diets

Figure A. Purina 5881 (+ and - indicates .1% Na added to diet or taken away from diet).

Figure B. Death Rate Purina 5881 and Plain Water.

Figure C. Purina 5775 .29% Sodium and Water.

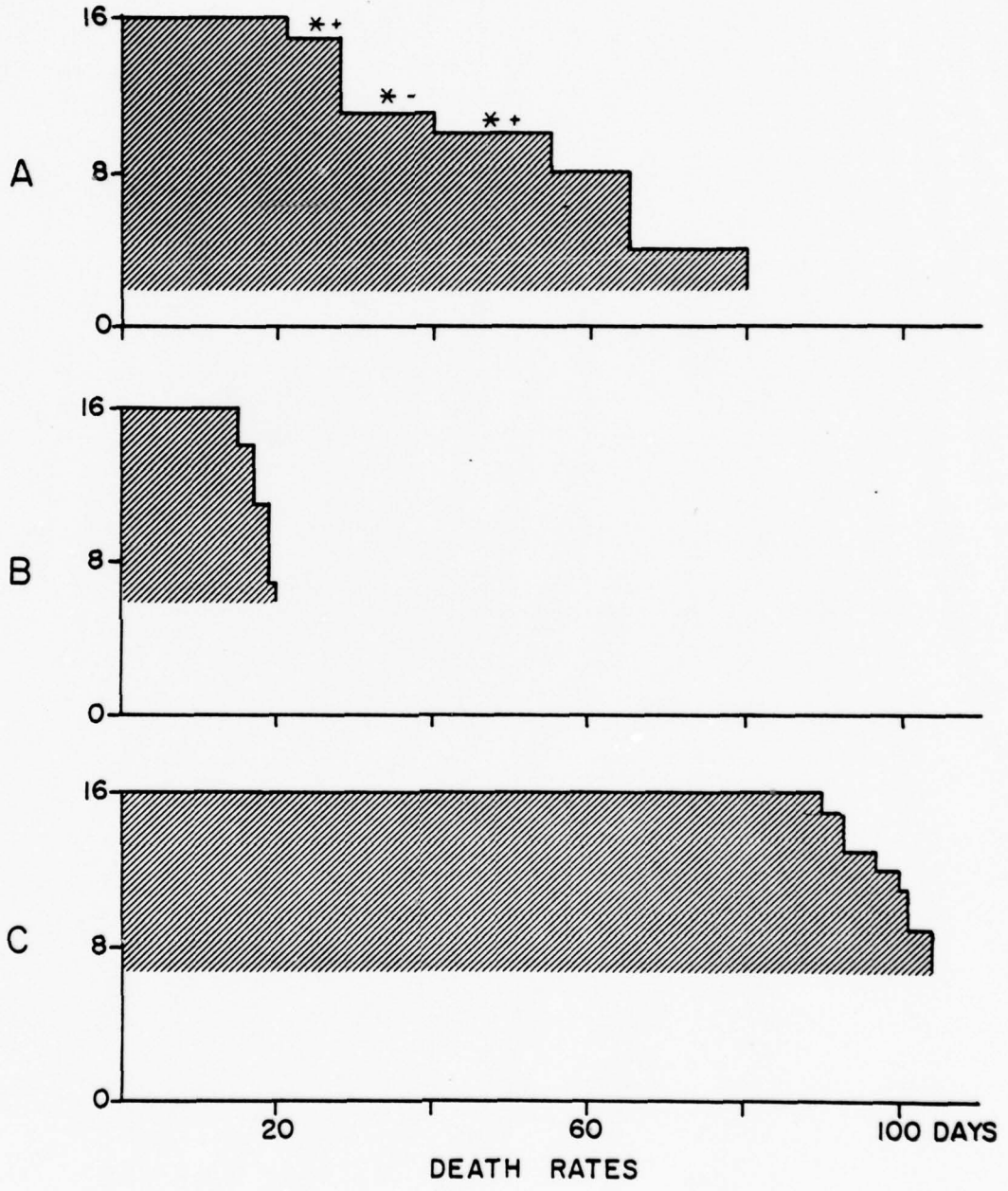
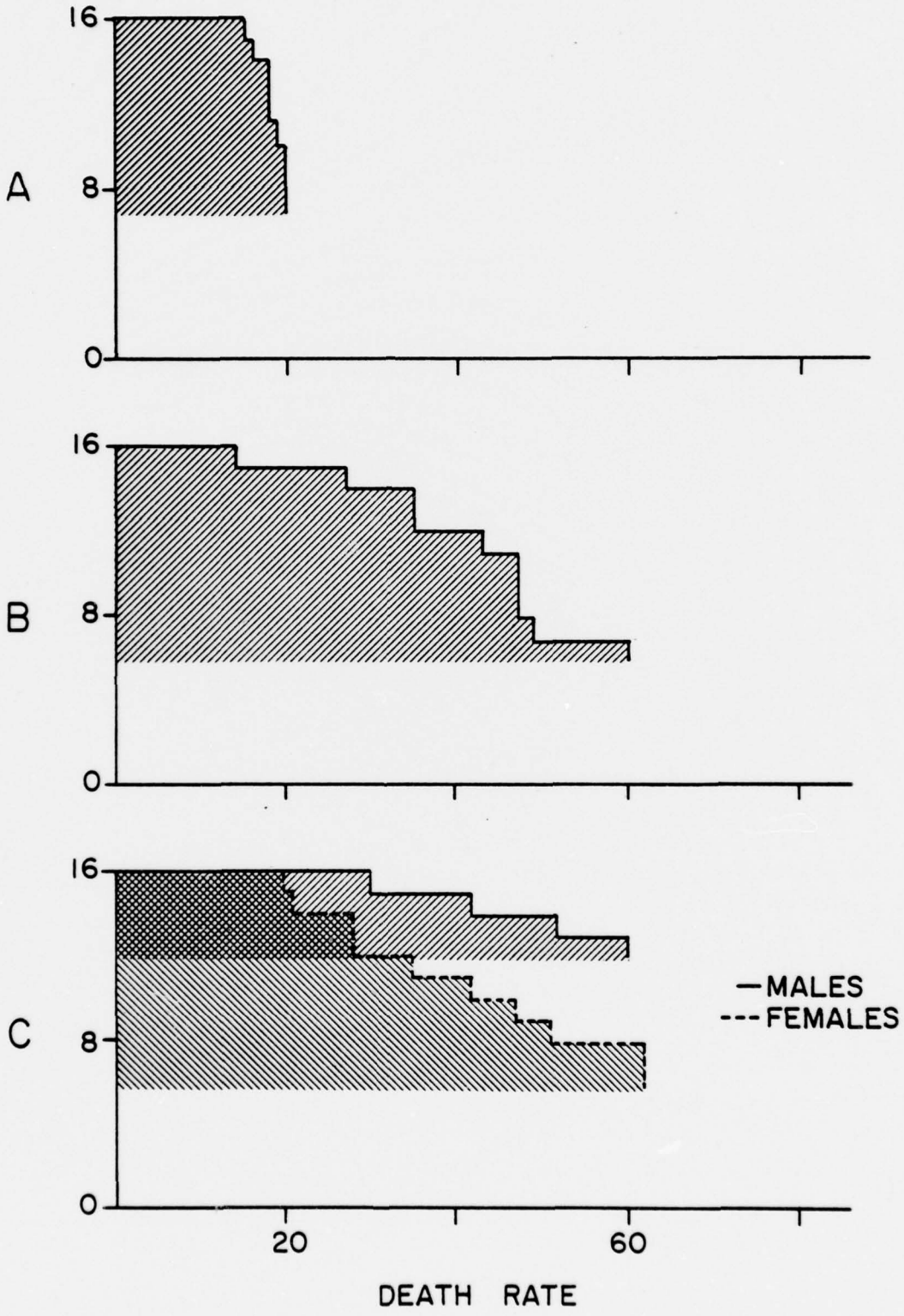

16
8
0
A
*+
*-
*+
16
8
0
B
16
8
0
C
20
60
100 DAYS
DEATH RATES

Figure 5.--Death Rates on Grain Mixture

Figure A. Death Rate on Grains and Plain Water.

Figure B. .3% Sodium in the Water and Grains.

Figure C. 0.08% Sodium in the Water and Grains.


16
A
8
0
16
B
8
0
16
C
8
0
20
60
DEATH RATE
—MALES
---FEMALES

DISCUSSION

Blood Pressure

Failure to obtain a pulsation in the tail during blood pressure measurements of mice on low salt diet may have been an expression of diminished sympathetic drive, especially to the sweat glands. Bradykinin released by the sweat glands was responsible for vasodilatation in the tail and the dissipation of heat. In mice on a low salt diet, this release was impaired as the result of the reduced sympathetic drive. Therefore, proper tail pulsation was absent for these reasons and thus precluded blood pressure measurement in these mice.

Hartroft Diet

The results of the Hartroft diet study made it clear that mice cannot survive long without sodium in their diets. They were unable to maintain body weight and soon died of malnutrition, despite the presence of food. The fact that boxed siblings or isolated mice on the Hartroft diet did not survive precluded further attempts to use it under situational stress.

Purina Test Diets

The results of the studies with Purina test diet No. 5881 showed that the diet was basically inadequate. Mice under stress either did not tolerate the low levels of sodium in the diet (17 mEq/day) or they did not maintain health because the composition was inadequate.

Perhaps the fact that the 60% carbohydrate of the diet was sucrose-dextrose rather than grain-based starch was part of the problem. Nevertheless, as Ralston Purina Company stated, the diet appeared to be adequate for the growth and maintenance of animals in a nonstressful environment.

A possible explanation of the massive cannibalism that occurred was that mice react with increased irritability when they do not feel well physically. As has been previously stated, mice in an intercommunicating box system fed on a standard Purina Lab Chow had a tendency to bite the tail and rump. But, they did not demonstrate the grossly aberrant behavior of gnawing on the upper back as was observed in the animals on a low salt diet. This suggested that mice under stress had an increased need for salt, and when their diet did not satisfy their salt hunger, they were driven to cannibalism after tasting the salt in the blood of the wounded mice. In the absence of open wounds on the males, it was not uncommon to find the

females cannibalized. Usually the smaller animals that were less able to defend themselves were the victims. Moreover, the less stressed nonsibling male mice (stress ±) in a box did not show this aberrent cannibalistic behavior, despite their low salt diet.

In the experiment with Purina test diet No. 5775 containing 0.29% sodium, both the boxed siblings (stress ±) and the isolated mice (stress 0) grew rapidly. Although they had the same dull coats and irritability as the mice in the colony, they were not stressed in competing for food or females and thus did not experience social breakdown or cannibalism.

Mixed Grain Diets

Two different types of grain diets were studied; one with 0.3% sodium and the other with 0.08% sodium added to the drinking water. This was to allow one group the equivalent in man of 10 grams of salt a day and the other group, 2 grams of salt a day.

The mice on the grain mixture with 0.3% sodium added (10 grams/day in man) had a steady, high death rate. The nonstressed controls in boxes had no deaths. It was concluded that the grain mixture diet containing additional sodium in the water was not nourishing enough to sustain mice under stress, but that it was sufficiently nourishing for nonstressed mice. There was a strong

possibility that this diet suffered from a gross lack of the essential amino acid lysine. This was borne out by the fact that the commercial diets, based on grain, always added fishmeal, which is high in lysine. The marginal quality of the diet affected the normal breeding patterns and the survival of young mice. Extensive nursing was required before young mice could be successfully weaned without dying. In an attempt to aid the inadequacies of the diet, vitamines were added to the drinking water, but no improvement was noticed.

The grain mixture diet with tap water (no sodium supplement) produced the same results as the low sodium Purina test diet No. 5881. It was not only nutritionally inadequate but also the deficiency in sodium may have triggered cannibalism in the mice as they tried to satisfy their hunger for salt and food.

When 0.08% sodium was added to the water in combination with the grain mixture, the sodium consumption was equivalent to 2 grams of salt in the daily diet of a 70-kilogram man. In man, this low salt intake represented the diet of a person who does not use commercially prepared foods or additional salt. This diet was perhaps the best one for keeping mice under stress in good health with one puzzling exception--many females died. Observations of females in breeder boxes on a grain diet suggested that

pregnancy may replace psychosocial stimulation as a stress, thus accounting for the high death rate after giving birth.

SUMMARY OF DATA PRESENTED

The original aim of this pilot study was to determine whether the low ingestion of salt could defer or prevent the onset of hypertension in mice. Tests showed that none of the available low salt diets were suitable for socially stressed mice. Therefore, our goal became the development of a diet sufficiently low in salt, yet nutritionally balanced.

Many of the early studies showed that a reduced sodium intake could achieve a reduction in blood pressure. If antihypertensive diuretic medications were not widely available today, the Kempner rice diet would be widely used. It effectively reduced the salt intake to half a gram a day or less; but is was suspected that the patient must not be psychosocially stressed if there was to be a reduction in blood pressure without unacceptably severe symptoms.

Reference to studies of populations showed that the normal salt intake of unacculturated peoples was not lower than 2 to 4 grams a day. Such people maintained normal blood pressures well into their sixties. No culture was

found in which people live on less than 1 gram of salt a day. According to the anthropologist Chagnon, even the Yanomamo Indians of Brazil and Venezuela had a daily intake of at least 1.5 to 2 grams of salt in the form of fish and meat.

It was shown that the inhabitants of New Guinea, who were primarily vegetarians eating little meat, developed a salt trade to compensate for a low salt diet. Such a trade did not flourish among hunting groups which ate moderate amounts of meat and thus got sufficient sodium from their diet.

The need for additional salt under stress was shown in a study on small wild rodents. They drank a saline solution instead of water when reacting to a stress which stimulated high adrenal cortical activity. They drank plain water when they were not stressed. It was also observed that the combination of high salt intake with high adrenal cortical activity increased the probability that hypertension would develop. In contrast, a low salt intake decreased sympathetic reactivity.

Sodium levels were shown to affect many areas of the blood pressure mechanism. A low salt intake desensitized the vascular bed by reducing its ability to store or to react to norepinephrine. Sodium levels also affected the renin-angiotensin system. During a low salt intake, the

levels of angiotensin II were high; yet, there was no concurrent pressor effect. At the same time, aldosterone increased helping to retain sodium. Meanwhile, the blood flow patterns of the kidney were also appropriately changed to a preference for the salt-saving tubules.

For this study, mice were placed in standard population cages to develop the necessary prolonged psychosocial stress. Basic techniques developed in this laboratory were used in maintaining them and in experimenting with the various diets and feeding systems.

The Hartroft diet was unsuccessful due to its lack of sodium. It would not maintain even unstressed mice (stress 0) and was eliminated. Purina test diets No. 5881 and No. 5775 were inadequate from the viewpoint of hypertensive studies because the mice could not successfully endure the necessary months of stress in the socially competitive colonies (stress +++). However, these diets did satisfy Ralston Purina's assertion that they would adequately maintain and allow for the normal growth of mice at stress levels below ++.

Certain human implications were drawn from the data thus far presented. It was seen that human studies would be difficult to implement because of the difficulties in adapting controlled stress and subjective measurements to human subjects. Although Dahl (16) stated that some of

his associates lived comfortably on as little as 3/4 to 1 gram a day salt intake without problems, we did not have a record of their ability to deal with psychosocial stress.

Realization of the great difficulty in maintaining a daily salt intake of even 2 to 4 grams in our society resulted in the only available choice of a higher salt intake. Data showed that a person that does not add salt to his food and does not cook with salt had a salt intake of 5 to 7 grams a day. However, there was a feasible alternative. The addition of a thiazide diuretic to this 5 to 7 gram intake reduces the body sodium level to the equivalent of 2 grams a day intake, and a lower blood pressure will result without further restriction of sodium.

The evidence suggested that the hypertension will benefit by reducing the salt in his diet to this level of approximately twice his maximum physiological needs rather than his taste preference. Attaining true physiological need of 2 grams a day presented a problem and today other avenues of relief from hypertension have been offered. Reduction of salt in the diet was only one of these and there were clear limits to its effectiveness. On less than 2 grams a day the individual was vulnerable both to heat stress and to psychosocial stimulation. Hence, a reduction to 5 to 7 grams of salt represented a practical limit for our society. Finally, now that the principles were worked

out, it remained to be seen at precisely what level of sodium intake psychosocially stimulated mice on an adequate low sodium, grain based diet would fail to develop hypertension and show a diminished severity of patho-physiological changes.

CONCLUSIONS

1. The minimum intake of salt for a nonstressed mouse was certainly no greater than the equivalent in man of 1.5 to 2 grams of salt a day.

2. An intake of salt below this level was not conducive to the health and well-being of mice in a psychosocially stressed colony (stress ++).

3. A special low sodium, grain based Purina diet was formulated to contain the equivalent of 3 to 4 grams of salt intake in a 70 kilogram man's daily diet. This diet expressed the practical minimum level for man and mouse under social stress. The comparison of the results obtained from this diet to those obtained from the diets in this study have produced a possible answer to the question of whether salt levels can influence the onset and intensity of hypertension.

4. It was believed that this pilot study has accomplished the necessary ground work for further investigations into the underlying problems of salt and blood pressure in mouse population colonies.

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